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Comparative cytogenetic analysis of the Chilean leptodactylid frog genus *Telmatobufo*, with the description of the chromosomes of *T. venustus*

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Abstract.—A comparative cytogenetic analysis of the Chilean frog genus *Telmatobufo* show that all the species share 2N = 26 and FN = 52. The chromosomes of *T. venustus* are described here for the first time. A polymorphic condition was detected on chromosome pair 3 of *T. bullocki*. Differences in C-band patterns and nucleolar organizer region (NOR) positions imply karyological differentiation in these species. Transformation of euchromatin to heterochromatin and loss of heterochromatin are hypothesized to have taken place in karyological evolution of some chromosomes in *Telmatobufo* species. Some NORs are considered as specific chromosome markers.

Leptodactylid frogs of the genus *Telmatobufo* (*T. australis*, *T. bullocki*, *T. venustus*) are a rare group (no more than 15 known specimens) endemic to the temperate *Nothofagus* forests of southern Chile (Formas 1979). Venegas (1975) described the karyotype of a single female of *T. bullocki*, and Formas & Pugin (1979) reported the chromosomes of *T. australis* from four tadpoles. Both taxa have a karyotype of 2N = 26 chromosomes. The karyotype of *T. venustus* has remained unknown until recently. The leptodactylid frogs of the subfamily Telmatobiinae mostly have karyotypes with 26 chromosomes, although some species have 22 or 34 chromosomes (Kuramoto 1990). Reig (1972) considered the formula 2N = 26 as primitive in the Leptodactylidae because its most primitive members (*Caudiverbera*, *Alsodes*, *Batrachyla*) have karyotypes with 26 chromosomes.

In this paper we describe for the first time the standard karyotype of *T. venustus*, and redescribe the standard karyotypes of *T. bullocki* and *T. australis*, all of them endemic telmatobiine frogs of central and southern Chile. Additionally, their C-banding pattern and NOR location were analyzed and compared in order to assess the extent of karyotypic divergence among these frogs, and to establish their karyological similarities.

Materials and Methods

Specimens examined.—The following specimens were used in this study: three females [IZUA (Instituto de Zoología Universidad Austral de Chile) 3159, 3161, 3164], two males (IZUA 3158, 3160), and six tadpoles (IZUA 3169) of *T. australis* (Chiveria, Valdivia Province, southern Chile: 40°08′S, 73°40′W). One male (IZUA 3157) and 26 tadpoles (IZUA 3147) of *T. bullocki* (Ruca-Pehuén, Arauco Province, southern Chile: 37°40′S, 73°25′W). One male (IZUA 3166) and one female (IZUA 3167) of *T. venustus* (Altos de Vilches, Talca Province, central Chile: 35°28′S, 71°11′W). All the specimens were collected by Lila Brieva, John Balladares, César Cuevas, J. Ramón Formes and José Núñez during the summer of 1998.

Chromosome preparations.—Chromo-

some spreads were obtained from duodenal epithelium using the technique described by Formas (1991). The chromosomes of the tadpoles were obtained from the epithelial cells of the tail using the method described by Bogart (1972). C-band patterns and NOR locations were determined by staining according to the techniques of Sumner (1972) and Rufas et al. (1982), respectively. Chromosome complements in several spreads from each individual were counted with a microscope and the best five chromosome spreads were selected for analysis. Centromeric positions were established according to Levan et al. (1964). Relative lengths of chromosomes were determined according to Bogart (1970). The secondary constrictions were not included in the measurements. Idiograms were constructed from the average measurements of ten homologous chromosomes from five well-spread cells.

Results

Standard karyotypes.—The description of the chromosomes of *T. venustus* is based on the analysis of 55 metaphase figures. The karyotype of *T. venustus* (Fig. 1c) has a diploid number of 2N = 26. All chromosomes are biarmed, so the fundamental number (FN) is 52. When chromosomes are arranged in pairs of decreasing length, pairs 1–4 are large (>100 units), pairs 5 and 6 are intermediate (between 80 to 100 units), and 7–13 small (<80 units). Pairs 1, 4, 7, 8, 9, 10, 11, and 13 are metacentric; 2 and 12 submetacentric; and 3 and 6 subtelocentric (Table 1). The short arms of pairs 5, 6, and 12 show secondary constrictions. In both the female and the male, one member of pair 5 has the secondary constriction much longer than its homologue. No sexual dimorphism was detected in the karyotype.

The redescription of the karyotype of *T. bullocki* is based on the analysis of 30 metaphase spreads. The karyotype of this species (Fig. 1b) consists of 26 chromosomes, all with two arms (FN = 52). A distinct gap in relative length is evident between 6 and 7; chromosomes are divided into three groups, large chromosomes (1–4), intermediate (5 and 6), and small chromosomes (7–13). Pairs 1, 4, 7, 8, 9, 11, and 13 are metacentric; pairs 2, 10, and 12 are submetacentric; and pairs 3, 5, and 6 are subtelocentric. Pair 12 has a secondary constriction in the short arms (Table 1). A polymorphic condition was detected on pair 3 (Fig. 1b). In the male, the chromosomes of pair 3 are different in size (3′ab), although similar in morphology (st). The same situation was observed in 14 (77%) tadpoles; the remaining six larvae (23%) had the chromosome pair 3 in homomorphic (3 aa) condition (both members similar in size). Secondary constrictions were observed on short arms of chromosome pair 12 and in one member of chromosome pair 3′ (b) (Fig. 1b).

The redescription of the chromosomes of *T. australis* is based on the analysis of 80 metaphase spreads. The karyotype of this species (Fig. 1a) has a diploid number of 26 chromosomes, all with two arms; its FN is 52. Chromosomes of *T. australis* form three size groups: large chromosomes (1–5), intermediate (6), and small chromosomes (7–13). Pairs 1, 4, 7, 8, 9, 10, 11, and 13 are metacentric; pairs 2 and 12 submetacentric; and pairs 3, 5, and 6 subtelocentric. Pairs 6 and 12 have a secondary constriction on the shorter arm (Table 1). No sexual dimorphism was detected in the karyotype.

C-banded karyotypes.—The C-banded karyotype of *T. venustus* (Fig. 2e) exhibits constitutive heterochromatin in the centromeric region of all chromosomes, except in chromosome pair 4. Telomeric C-bands occurred on the long and short arms of chromosome pairs 1, 2, 4, 8, 10, 11, and 12; pairs 3, 6, and 9 present only telomeric C-bands (long arms). A polymorphic condition was detected in the C-banded pattern of chromosome pair 5. One member (5a) presents a marked telomeric band on the short arms, which is absent on its homo-

Table 1.—Relative length, arm ratio (mean and standard deviations), and types of chromosomes (m = metacentric; sm = submetacentric; st = subtelocentric) of *Telmatobufo australis, Telmatobufo bullocki,* and *Telmatobufo venustus.*

	T. australis			*T. bullocki*			*T. venustus*		
Pair N°	Relative length	Arm ratio	Type	Relative length	Arm ratio	Type	Relative length	Arm ratio	Type
1	159.91 ± 6.87	1.22 ± 0.10	m	163.84 ± 0.42	1.35 ± 0.10	m	167.55 ± 3.47	1.25 ± 0.09	m
2	123.78 ± 8.31	2.05 ± 0.27	sm	129.74 ± 3.04	1.87 ± 0.17	sm	124.77 ± 11.45	2.13 ± 0.26	sm
3	113.08 ± 4.57	3.07 ± 0.19	st	125.44 ± 0.84	3.36 ± 0.15	st	111.40 ± 7.95	3.85 ± 0.55	st
3′ a				125.26 ± 2.85	3.42 ± 0.25	st			
3′ b				*107.10 ± 2.43	5.50 ± 0.12	st			
4	105.06 ± 7.57	1.33 ± 0.07	m	104.31 ± 2.12	1.38 ± 0.08	m	106.68 ± 4.86	1.42 ± 0.12	m
5	100.32 ± 4.61	2.67 ± 0.25	st	95.58 ± 5.44	3.14 ± 0.12	st	* 94.38 ± 5.45	6.80 ± 0.58	st
6	* 87.32 ± 4.80	5.50 ± 0.03	st	80.27 ± 3.67	3.95 ± 0.13	st	* 91.77 ± 7.01	6.00 ± 0.69	st
7	52.99 ± 2.50	1.41 ± 1.41	m	54.83 ± 5.30	1.13 ± 0.14	m	52.35 ± 1.60	1.35 ± 0.11	m
8	49.64 ± 2.12	1.27 ± 0.62	m	50.50 ± 3.53	1.60 ± 0.57	m	47.04 ± 2.37	1.22 ± 0.09	m
9	48.74 ± 1.47	1.11 ± 0.05	m	48.25 ± 1.90	1.24 ± 0.05	m	44.92 ± 5.50	1.88 ± 0.12	sm
10	42.61 ± 3.32	1.20 ± 0.09	m	43.85 ± 4.31	2.10 ± 0.60	sm	40.26 ± 1.35	1.23 ± 0.24	m
11	40.05 ± 1.79	1.45 ± 0.26	m	42.76 ± 3.74	1.54 ± 0.24	m	38.51 ± 2.33	1.30 ± 0.24	m
12	* 39.33 ± 1.86	2.00 ± 0.21	sm	* 38.36 ± 0.63	2.60 ± 0.11	sm	* 36.48 ± 2.35	2.01 ± 0.03	sm
13	35.72 ± 1.39	1.40 ± 0.27	m	33.85 ± 4.31	1.23 ± 0.17	m	32.88 ± 3.30	1.22 ± 0.12	m

* Chromosomes with secondary constrictions.

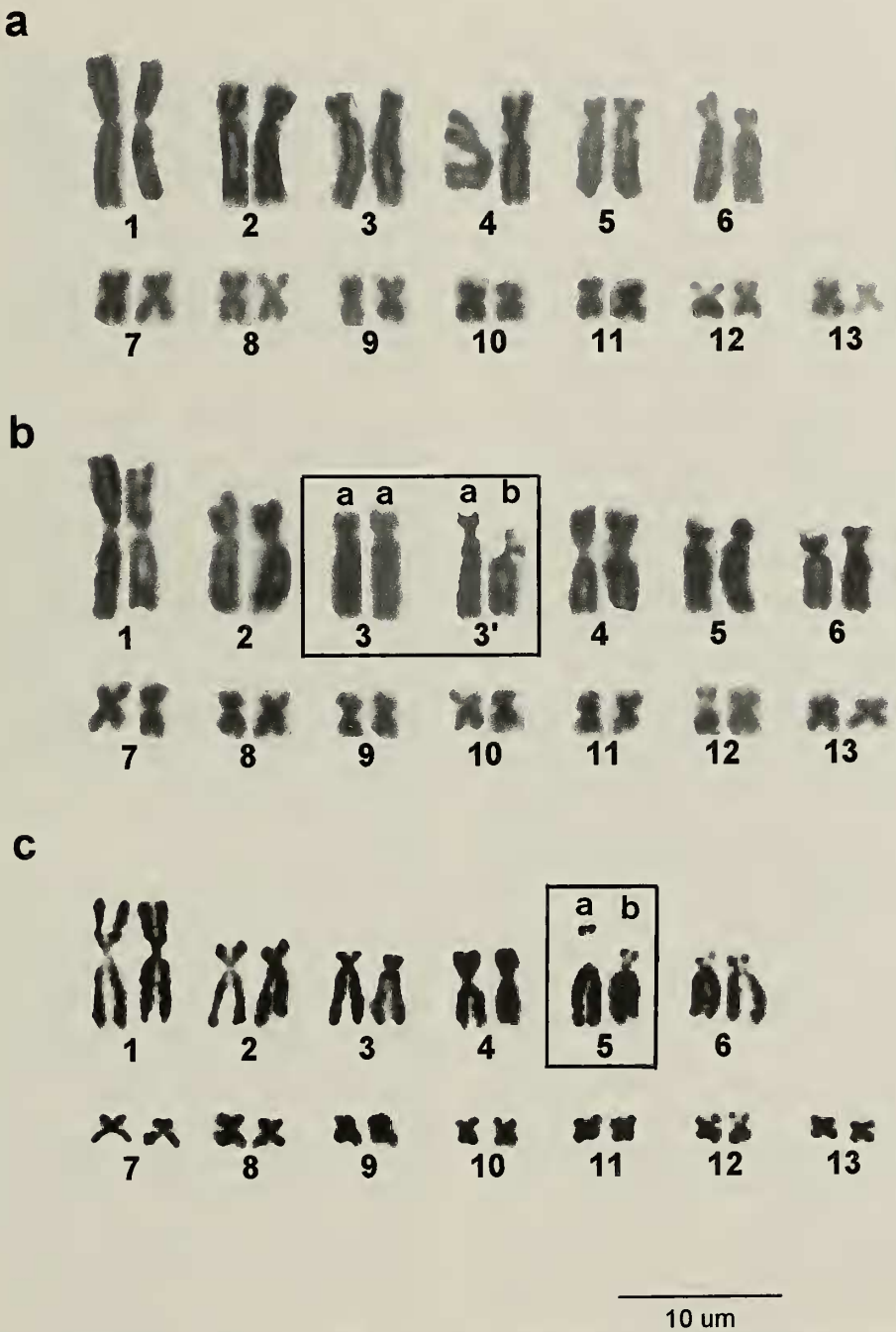

Fig. 1. (a) Standard karyotype of a male of *Telmatobufo australis*, (b) standard kartyotype of a male of *T. bullocki*; the polymorphic condition (aa,ab) detected in the tadpoles (pair 3) is framed, (c) standard karyotype of a male of *T. venustus*; the frame indicates the difference in size of the secondary constriction detected between both member of pair 5.

logue (5b). The polymorphic condition observed in chromosome pair 5 was detected in both female and male. Interstitial heterochromatin was observed in the long arms of pairs 1, 2, 3, and 6. The C-banded idiogram of *T. venustus* is presented in Fig. 3c.

In the C-banded karyotype of *T. bullocki* (Fig. 2c) small heterochromatic bands were observed in the centromeric region of chromosome pairs 2, 3-3′, 4, 5, 6, 8, 9, 10, 11, 12, and 13; in chromosome pair 7 the evidence for heterochromatic bands is faint, and it is absent in chromosome pair 1.

Telomeric bands were observed in chromosome pairs 1, 3′b, 4, and 5 (both arms), 2, 3a, 3′a, and 6 (long arms). An interstitial C-band was observed on the long arm of chromosome pair 6. The small chromosomes (7–13) do not show telomeric C-bands. The C-banded idiogram of *T. bullocki* is shown in Fig. 3b.

The C-banded karyotype of *T. australis* (Fig. 2a) shows well-defined bands in the centromeric regions of chromosome pairs 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, and 13; in chromosome pairs 5 and 6 the bands are strongly stained. The stained bands are less marked in chromosome pairs 4 and 12. Pairs 1–4 exhibit telomeric heterochromatic bands (both arms); however, telomeric bands were not observed on the small chromosome pairs (7–13). Chromosome pairs 5 and 6 have no telomeric bands on the short arms. Pairs 3, 5, and 6 have interstitial blocks of constitutive heterochromatin on the long arms. The C-banded idiogram of *T. australis* is shown in Fig. 3a.

Nucleolus organizer regions (NORs).— Silver staining of chromosomes showed the NORs to be located within the secondary (nucleolar) constrictions of the short arms of chromosome pairs 6 and 12 (*T. australis*, Fig. 2b), 12 (*T. bullocki*, Fig. 2d), and 5 and 12 (*T. venustus*, Fig. 2f). A tandem duplication was observed in heterozygotic condition on chromosome pair 5 of *T. venustus* (Fig. 2f).

Discussion

The karyotype of *T. bullocki* as described by Venegas (1975) based on a single female, is only partially congruent with that obtained by us. Firstly, an evident secondary constriction on the short arms of pair 12 (Fig. 1b) was observed here. Second, chromosome pair 3 was subtelocentric, not metacentric, and third, chromosome pair 4 was metacentric, not subtelocentric as previously described by Venegas. Finally, chromosome pair 13 was submetacentric here, not metacentric. Venegas (1975) described chromosome pair 3 as homomorphic (both homologues similar in size); this condition was here reported in six tadpoles, although not in the male analyzed. The former descriptions of the karyotype of *T. bullocki* (Venegas 1975) and the polymorphism here reported for chromosome pair 3 (homologues different in size), suggest the existence of heteromorphic sex chromosomes in *T. bullocki*. This possibility should be tested by analysis of meiotic plates of males and additional mitotic plates of females.

The karyotype of *T. australis* as described by Formas & Pugin (1979) differs in part from our results. Secondary constrictions were observed on pairs 6 and 12; Formas & Pugin (1979) described those karyological features on chromosome pairs 3, 6, and 7. Chromosome pair 3 was submetacentric, not metacentric as previously described. In addition, chromosome pair 5 was metacentric, not subtelocentric. Finally, chromosome pairs 5 and 12 were subtelocentric and submetacentric, respectively, not metacentric as previously described.

The comparison of the standard karyotype of the three species of *Telmatobufo* showed that they do not differ notoriously with respect to the centromeric index; however, the small chromosome pair 10 is submetacentric in *T. bullocki*, although metacentric in *T. australis* and *T. venustus*, and the chromosome pair 9 is submetacentric in *T. venustus*, but metacentric in *T. australis*

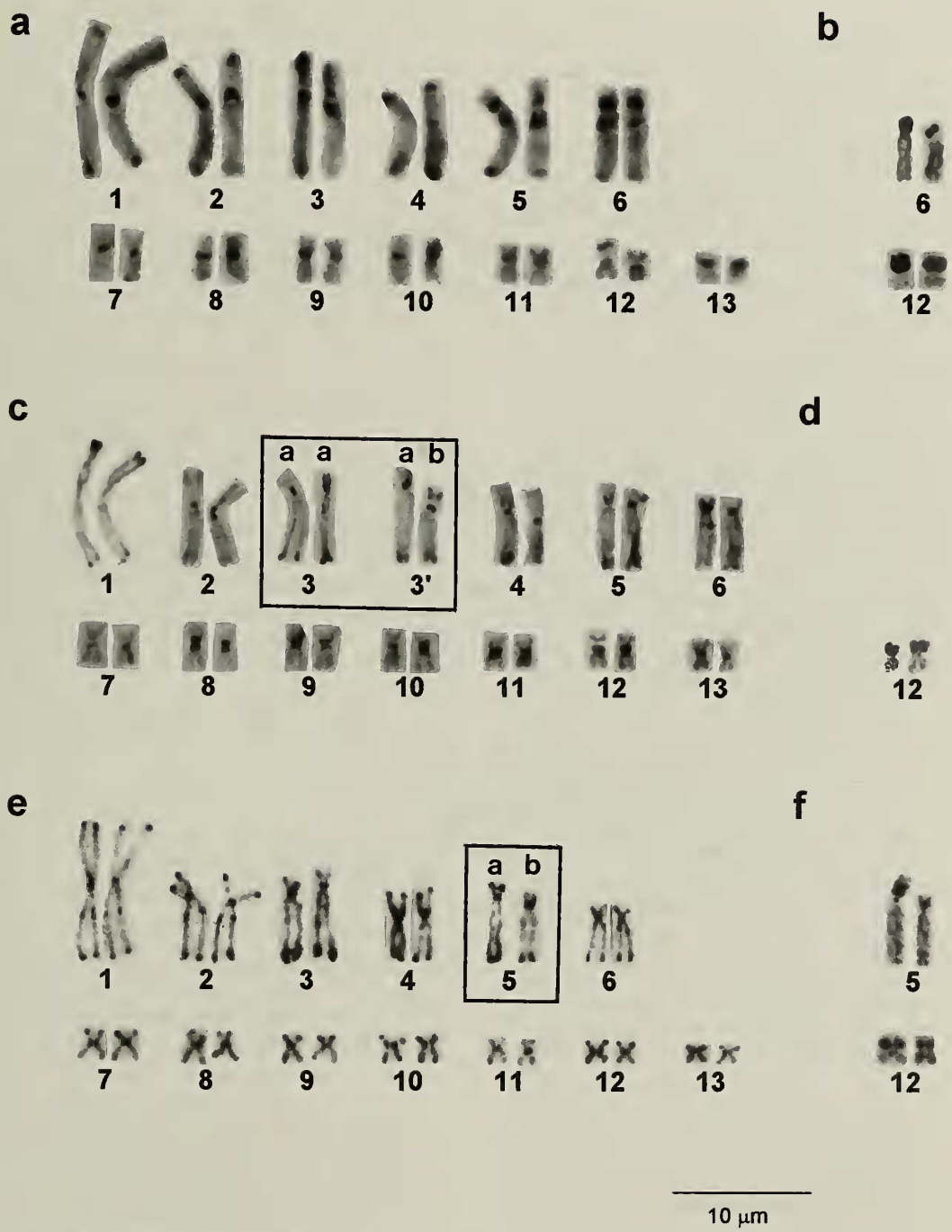

Fig. 2. (a) C-banded karyotype of a male of *Telmatobufo australis*. (c) C-banded karyotype of a male of *T. bullocki*; the polymorphic condition (aa,ab) detected in the tadpoles (pair 3) is framed. (e) C-banded karyotype of a male of *T. venustus*; the frame shows both member of pair 5, they are different in sizes and C-banding patterns. Positions of the NORs in *Telmatobufo* species; (b) pairs 6 and 12 (*T. australis*), (d) pair 12 (*T. bullocki*), (f) and pairs 5 and 12 (*T. venustus*). A tandem duplication is observed in one homologue of pair 5 of *T. venustus*.

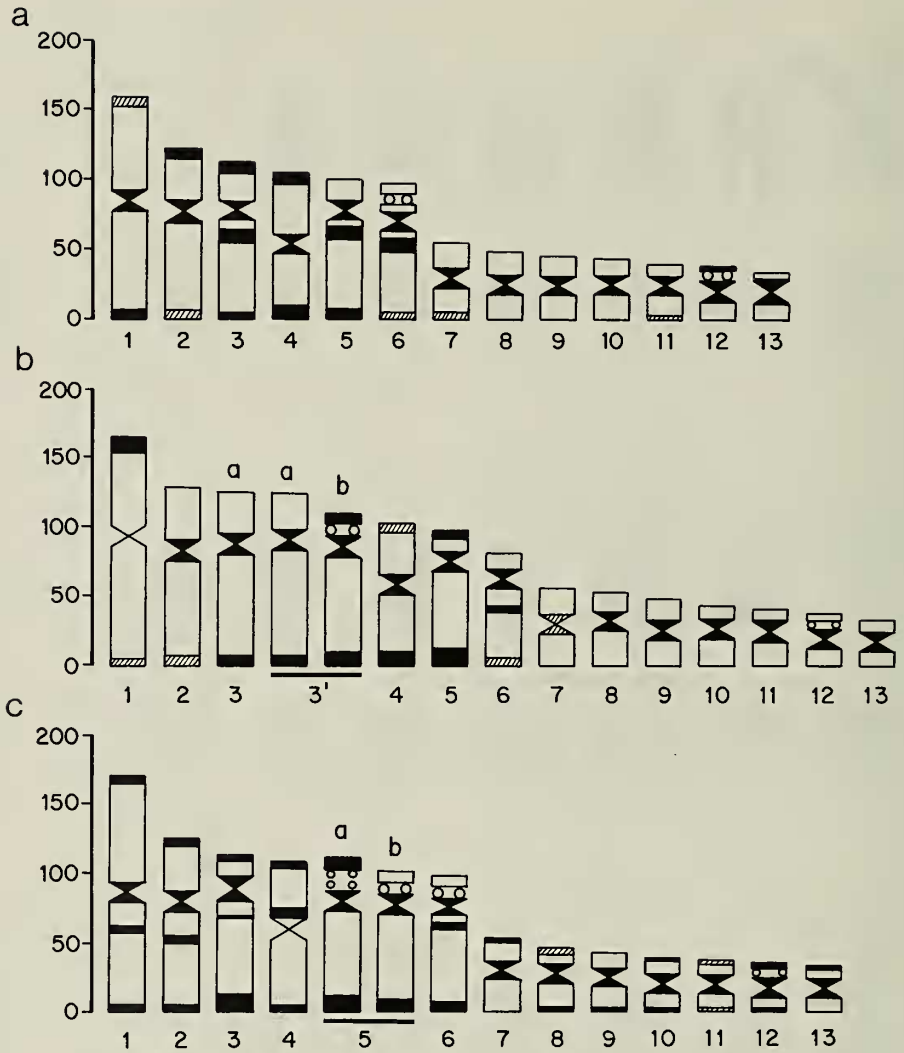

Fig. 3. (a) C-banded idiograms of *Telmatobufo australis*, (b) C-banded idiograms of *T. bullocki*; the bar indicates the polymorphic condition (pair 3) detected in tadpoles, (c) C-banded idiograms of *T. venustus*; the bars shows the differences in sizes and C-banding patterns detected in pair 5. The scale from 0 to 200 refers to the normalized length of the chromosomes relative to the total complement length.

and *T. bullocki*. In terms of number (2N = 26) and relative size the karyotypes of *Telmatobufo* species are very similar; however a minor difference was detected. Pair 5 is large in *T. australis*, but intermediate in *T. bullocki* and *T. venustus*.

In contrast to the relative morphological uniformity of the standard karyotypes, important differences were observed in the amount of constitutive heterochromatin, as well as in the position and size of C-bands.

The constitutive heterochromatin of anuran chromosomes presents a highly variable pattern of distribution, in qualitative as in quantitative respects (Schmid 1978a, 1978b; King 1980), and the C-bands have been used to differentiate otherwise closely similar standard karyotypes (Miura 1995, Spasić-Bosković et al. 1997). In most anuran species, the nucleolar organizer regions (NORs) occupy an interstitial or subterminal position along the chromosomes,

and interspecific comparisons have shown that the Ag-stained NORs are almost always found in the same chromosomal region in karyotypes of closely related species (Schmid 1982).

The C-banded karyotypes and idiograms of *T. australis*, *T. bullocki*, and *T. venustus* (Figs. 2 and 3) show that *T. australis* and *T. bullocki* share a similar C-band pattern (centromeric bands) on their small chromosome pairs (7–13). In *T. bullocki*, the centromeric band on chromosome pair 7 is less marked. *Telmatobufo venustus* differs from *T. australis* and *T. bullocki* by having a telomeric band on chromosome pairs 7–13 (both arms of 8, 10, 11, and 12, short arms of 7 and 13, and long arm of 9). The three species share an interstitial band on the long arm of chromosome pair 6. In *T. australis*, this band is better developed than in the other species. *Telmatobufo australis* can be differentiated from its congeners by the existence of interstitial large bands of heterochromatin in the long arm of chromosome pairs 3 and 5. On the other hand, the centromeric bands of this species are better developed than in *T. bullocki* and *T. venustus*. *Telmatobufo venustus* is distinguished from *T. australis* and *T. bullocki* by possessing a strong telomeric C-band on the long arm of chromosome pair 3 and interstitial bands on the long arms of chromosome pairs 1 and 2. *Telmatobufo bullocki* differs from its congeners by the absence of centromeric bands on chromosome pair 1.

Transformation of euchromatin to heterochromatin (King 1980, 1991) and loss of heterochromatin, especially in sex chromosomes (Iturra & Veloso 1991, Cuevas & Formas 1996) are processes apparently implicated in the evolution of anuran genomes. The C-banding patterns of *Telmatobufo* species are rather complex and difficult to interpret, and the evolutionary mechanisms cited above are only used to explain the heterochromatin evolution of some of the chromosomes. Frogs in the species group constituting the genus *Telmatobufo* share the same basic karyotype in terms of gross morphology. This is emphasised by each of the species sharing an interstitial band on the long arm of chromosome pair 6, and strong centromeric bands on all chromosomes, except for chromosome pair 1 in *T. bullocki* (band absent) and chromosome pair 4 in *T. australis* (weak band). The absence of a centromeric C-band in chromosome pair 1 of *T. bullocki* appears to be a derived condition, because the C-banded karyotype of the monotypic genus *Caudiverbera* [*C. caudiverbera*: sister group of the genus *Telmatobufo*, (Lynch 1978)] has centromeric C-bands on all chromosomes (Veloso 1977), and for this reason, we assume loss of heterochromatin.

Transformation of euchromatin to heterochromatin is here postulated to explain the presence of interstitial heterochromatin bands in the long arms of chromosome pairs 1 and 2 of *T. bullocki* due to their absence in the C-banded karyotypes of *C. caudiverbera* (Veloso 1977). Chromosome pair 6 in *T. venustus* and a member of chromosome pair 3 (3′b) in *T. bullocki* show a conspicuous secondary constriction; however, they are negative to Ag-staining. Lack of Ag-staining in these chromosomes can be explained by inactivity of their NORs in the preceding interphase (Schmid 1982).

The presence of NORs in chromosome pair 12 of all three *Telmatobufo* species is one example of the strong similarities among their karyotypes. This feature, along with the presence of centromeric C-bands on all chromosomes and the interstitial C-bands on the long arm of pair 6, provides cytological characters that can be used in the chromosomic diagnosis of the genus, previously based only on osteological (Lynch 1978) and larval characters (Formas 1988). NORs on chromosome pair 6 of *T. australis* and chromosome pair 5 of *T. venustus* are interpreted as specific characters, and could be considered as chromosome markers for these taxa.

Karyotypes with 26 bi-armed chromosomes have been postulated by Reig (1972) as the primitive condition for members of

the family Leptodactylidae. *Caudiverbera caudiverbera*, one of the most primitive members of this group, with fossils reported from the Eocene-Oligocene of Argentinean Patagonia (Shaeffer 1949, Gasparini & Báez 1975, Báez & Gasparini 1975), presents a karyotype with 26 bi-armed chromosomes (Formas & Espinoza 1975). Using an immunological approach (microcomplement fixation), Núñez & Formas (2000) pointed out that *Telmatobufo* and *Caudiverbera* (the unique genera of the Calyptocephalellini tribe, Lynch 1978) diverged 35 million years ago (Lower Oligocene). The presence of 26 bi-armed chromosomes in *Telmatobufo* and *Caudiverbera* suggests that both genera share an old and primitive karyotype, which constitutes new evidence in support of Reig's hypothesis.

Among the telmatobiine frogs of the temperate *Nothofagus* forests of southern Chile, the primitive 26 bi-armed karyotype has been reported in the genera *Alsodes* [*A. monticola*, *A. tumultuosus*, *A. vanzolinii*, *A. verrucosus*, *A. gargola* (Formas & Vera 1983), *A. australis* (Formas et al. 1997), *A. kaweshkari* (Formas et al. 1998)], *Batrachyla* (*B. antartandica*, *B. leptopus*, *B. taeniata*), *Hylorina* (*H. sylvatica*), and *Insuetophrynus* (*I. acarpicus*) (Barrio & Rinaldi de Chieri 1971). If the karyotype with 26 chromosomes is interpreted as primitive, the presence of 22 chromosomes in *Alsodes nodosus* (Bogart 1970), and 34 chromosomes in *A. barrioi* (Veloso et al. 1981), can be considered a derived condition. As the primitive karyotype does not include telocentric chromosomes, the presence of telocentric chromosomes in *B. taeniata*, *B. antartandica* (one pair) and *A. barrioi* (five pairs) is also postulated to be a derived character. Species of the genera *Eupsophus* (Formas 1978, 1980, 1991; Cuevas & Formas 1996), *Eleutherodactylus* and *Syrrophus* (Bogart 1970) with chromosome numbers both higher and lower than 22 and 26, and numerous telocentric chromosomes, must be interpreted as examples of exceptional mechanisms of chromosome evolution within the Leptodactylidae family.

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